B2

--46. The protein of claim 1, wherein said protein is produced in a eukaryotic host cell.

47. The protein of claim 1, wherein said protein is produced in a mammalian host cell.

48. A mammalian host cell comprising the vector of claim 5.

49. The method of claim 41, wherein the host cell is a eukaryotic cell.--

REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.112, are respectfully requested.

By the present amendments, independent claims 1, 12, 19 and 41 have been amended to recite that the recombinantly expressed L1 major capsid protein is capable of substantially reproducing the antigenicity of intact papillomavirus virions.

Claims 25 and 26 have been amended as suggested by the Examiner.

Claims 46-49 are added which provide for the L1 protein or fragment to be expressed in eukaryotic or mammalian host cells. Support for these amendments may be found, e.g., in

the examples which disclose expression of the HPV-1 L1 protein in mammalian (cos) cells.

Turning now to the Office Action, claims 1-26 and 41-45 are under active consideration by the Examiner. Claims 27-40 are withdrawn from consideration as being directed to a non-elected invention. Although the restriction requirement is still respectfully traversed for the reasons set forth in the Election dated January 19, 1993, non-elected claims 27-40 are cancelled herein without prejudice or disclaimer to expedite prosecution.

Claims 1-26 and 41-45 have been rejected under 35 U.S.C. §112, second paragraph.

Specifically, claims 1-26 and 41-45 are stated to be vague and indefinite in reciting that the recombinant L1 or antigenic fragment is capable of reproducing the antigenicity "of all proteins found" on the intact papillomavirus. The Examiner indicates that the meaning of the intended proteins is thus unclear.

In response thereto, please note that applicants intend to claim any L1 protein or antigenic fragment thereof, produced by recombinant methods, which, by virtue of the recombinant host cell in which it is expressed, assumes the correct conformation, i.e., the conformation of the L1 protein as it is expressed in an intact papillomavirus virion and which therefore substantially reproduces the antigenicity thereof. The L1 protein comprises the major capsid protein of the papillomavirus which encapsidates the papillomavirus and

is expressed on the surface of the virus. Moreover, the immunological data herein indicates that the L1 protein is the protein which substantially (or entirely) mediates antigenicity in a papillomavirus infected host.

However, since the papillomavirus comprises other proteins, e.g., the L2 protein (the minor capsid protein of the papillomavirus), which may possibly mediate some antigenic response in a host, (although the disclosed data would suggest otherwise) claims 1, 12, 19, and 41 have been amended to recite that the recombinant L1 protein substantially reproduces the antigenicity of intact papillomavirus. It is accordingly believed that the meaning of the intended L1 proteins and antigenic fragments is clear from the subject application and the above remarks.

Claims 4-9 were rejected for being unclear as to whether the claimed vector contains a DNA or a protein. It is believed that this rejection has been obviated by the preliminary amendment dated January 19, 1993, wherein claim 4 was amended to recite that the vector comprises a DNA encoding the [L1] protein of claim 1. Therefore, the vector comprises a DNA sequence which upon expression results in a L1 major capsid protein or an antigenic fragment thereof.

Claims 11, 17, 18 and 21 were rejected for failing to use Markush phraseology. In response thereto, it is respectfully argued that Markush claim phraseology is not required claim phraseology. Rather, applicants are permitted to use any claim language which unambiguously recites what elements are

necessary to practice, and thus infringe the claims. This requirement is satisfied by the subject claims. For example, claim 11 is clearly directed to a recombinantly produced L1 protein which is either HPV16 or HPV18. Claim 17 is directed to a vaccine which comprises both the HPV16 and HPV18 L1 proteins. Claim 18 provides for a vaccine which contains the L1 protein of any of HPV1, 2, 3a, 4, 5, 6b, 7, 8, 9, 10, 11a, 12, 13, 16, and 18. Finally, claim 20 claims a method for protecting an animal against papillomavirus by administering an L1 protein as claimed in claim 19 which is selected from HPV1, 2, 3a, 4, 5, 6b, 7, 8, 9, 10, 11a, 12, 13, 16, and 18. Therefore, claims 11, 17, 18, and 21 are definite as written.

Claims 1-16 and 41-45 were also stated to be indefinite in failing to recite the size of the L1 antigenic fragments. This rejection has been addressed to some extent, supra. Essentially, an antigenic fragment simply refers to any L1 protein fragment which, by virtue of the host in which it is expressed, assumes the proper conformation, i.e., the conformation that the L1 protein assumes in intact papillomavirus virions. That is to say the antigenic fragment must be capable of reproducing or presenting to a host immune system the same conformational epitopes which are present on the L1 protein and which are expressed on the surface of intact papillomavirus virions.

Accordingly, since these epitopes are not contiguous on the L1 protein, but rather are spaced at different positions on the L1 protein, the claims do not read on small peptides,

e.g., of 7 or 20 amino acids as asserted in the Action. Moreover, the antigenic fragments are not of indefinite scope since the entire L1 protein is only about 55kd, and the claimed L1 fragment comprises an antigenic fragment thereof.

Finally, claims 25 and 26 are stated to be indefinite in failing to recite "at least one of HPVs". These claims have been amended herein in accordance with the Examiner's helpful suggestions.

Based upon the above arguments, withdrawal of the outstanding §112, second paragraph rejection of claims 1-26 and 41-45 is respectfully believed to be in order and is earnestly solicited.

Turning now to the §101 utility rejection, claims 15-18 and 20-26 stand rejected as allegedly lacking patentable utility.

The Office Action indicates that the disclosed data pertaining to the control of BPV-1 using sera from various hosts is inadequate to establish that the claimed L1 proteins will prevent papillomavirus infection in humans. In support of this rejection, the Examiner indicates that when animal or in vitro data is presented to establish utility, that there must exist an art recognized model to establish that these tests are accepted in the art. In re Hartop, 311 F.2d 249, 135 USPQ 419 (CCPA 1962). This rejection is respectfully traversed.

The subject application contains convincing evidence using two different assay systems, i.e., xenograft assays and

C127 cell assays, that BPV-1 infection may be neutralized by antiserum prepared using conformationally correct intact BPV-1 or BPV-2 virions as immunogens, whereas antisera obtained using denatured BPV-1 particles as immunogens was ineffective, i.e., it did not result in the reduction of cyst size. These assays and the results thereof are described in great detail in Example 1. Given that the results of both assays were in substantial agreement, it is reasonable to conclude that these assays are valid predictors of the capability of conformationally correct L1 proteins to induce the production of neutralizing antibodies and to thereby provide protection against papillomavirus infection.

Moreover, the application contains convincing evidence that L1 proteins, when expressed in suitable host systems, e.g., mammalian cells, are obtained in proper conformation, i.e. a conformation which presents the conformational neutralizing, epitopes to a host's immune system and which proteins are therefore capable of substantially reproducing the antigenicity of the intact papillomavirus virions.

In particular, the HPV-1 L1 protein, when expressed in cos cells, resulted in a full length L1 protein which reacted with four different monoclonal antibodies which specifically recognize conformational epitopes on HPV-1. This is shown by both ELISA and western blotting assays. (In contrast, L1 proteins which are expressed in bacteria lack native conformation, and do not induce antibodies which recognize or neutralize BPV-1 virions (Xin et al, Intervirology, 31:345-357

(1990); and Ghim et al, Int. J. Cancer 44:285-289 (1991)). As discussed in the application, e.g., at page 42, and as further shown by Example 1, the ability of antibodies to neutralize papillomaviruses is directly related to their ability to react with type-specific conformational epitopes on the virion surface. Indeed, previous studies have demonstrated that the predominant antibody response detected against HPV-1 in humans is directed against such conformational epitopes (Steele et al, Virology 174:388-398 (1990), Anisomova, et al, J. Gen. Virol. 71:419-422 (1990).

It would appear from the rejection that the Examiner has not found this data to be persuasive because in vitro and animal tests were utilized rather than clinical human testing.

However, the absence of such data should not defeat the patentability of the subject claims. As those versed in the papillomavirus art are well aware, it is totally unfeasible at this time to investigate in humans the antigenic response against HPV and its role in the progression of HPV mediated malignancy. However, this does not prevent the antigenic characterization of this virus. Instead, there are well established in vitro and animal models which are currently used for effectively studying papillomavirus infection. These models include, e.g., the canine oral papillomavirus (COPV) model for mucosal infection and BPV-1 based in vitro models. BPV-1, in fact, comprises the predominant art recognized model which is currently used to study virions of the PV genus. Dvoretzsky et al, 1984, teaches e.g., that BPV-1 infection of

murine fibroblasts comprises the best available in vitro model for studying neutralization of PV infection. Further, Christensen et al, (1990) 64:5678-5681, cited against the subject claims teaches that intact virions of BPV-1, HPV-11 and cotton tail rabbit papillomavirus (CRPV) protect against formation of papillomas/condylomas in the xenograft animal model system. Additionally, Ghim et al, Int. J. Cancer, (1991) 48, 280, 296, teaches that there is almost perfect agreement between BPV-1 induced neutralization in the murine C127 cell model and the fetal bovine skin xenograft model. Therefore, the literature establishes that the disclosed in vitro assays using BPV-1 are accepted in the art, and that they reliably predict the ability of papillomavirus proteins to induce virus neutralization.

Moreover, the role of conformational dependent antibodies on the induction of immunity to papillomaviruses is also accepted in the art and has further been demonstrated in the application. Specifically, the application contains convincing evidence that the HPV-1 protein expressed in cos cells is conformationally correct and that this protein binds to conformationally specific HPV-1 neutralizing antibodies. Moreover, Ghim et al, (1991) Id., teaches that bovine sera produced against non-conformational epitopes such as a recombinant beta gal BPV-1 L1 fusion protein of Pilacinsky et al, Bio/Technology, 2:356-360, (1984), and rabbit sera produced against denatured BPV-1 capsids does not neutralize BPV-1 infection of C127 cells or transformation of fetal

bovine skin xenografts. In contrast, cattle and rabbits immunized with intact BPV-1 virions produce neutralizing antibodies that protect C127 cells and the xenograft system against infection and/or transformation by BPV-1 virions. Evidence that the bovine host can elicit an antibody response specific to BPV-1 conformational epitopes is further reflected in the fact that antisera recognizing BPV-1 conformational epitopes provides substantially greater protection than antibodies recognizing linear epitopes; and moreover provides for substantially greater titers of neutralizing antibodies. Ghim et al, (1991), Id., further teaches that effective humoral responses against papillomavirus infections (which includes HPV infections) must be directed against conformationally-correct epitopes on the surface of PV virions.

Additional evidence of the critical importance of conformational dependent antibodies against PV and viral immunity is provided by a recent grant application by Richard Schlegel, a copy of which is included in the Information Disclosure Statement filed concurrently herewith. This reference relates to the study of COPV infection in beagle dogs which naturally express oral papillomas, and to the eventual design of a vaccine using conformationally correct L1 proteins. This animal model is highly relevant to human papillomavirus vaccine design since the COPV and HPV are very similar in structure. Note in particular, page 32 of this

grant wherein the sequences of the COPV L1 protein and the HPV-1 L1 protein are compared.

This reference contains preliminary data which indicates that inoculation of beagle dogs with COPV wart extracts (which comprise the intact L1 protein on their surface) can successfully protect said dogs from COPV infection. While this reference does not teach administration of a conformationally correct L1 protein by itself, it provides still additional evidence that conformationally correct papillomavirus proteins (from yet another papillomavirus strain) may induce protection against PV infection.

Still further evidence of the utility of conformationally correct L1 capsid proteins as effective immunogens is provided by a recent article by Kirnbauer et al, Proc Natl Acad. Sci., (1992), 89:12180-12184 which reference is included in the concurrently filed Information Disclosure Statement. Kirnbauer teach expression of the HPV16 and BPV1 L1 genes in baculoviruses. The conformationally correct proteins spontaneously assembly into capsid-like structures exhibiting an immunogenicity comparable to the intact papillomavirus virions. Moreover, the proteins provide for neutralizing antibody titers which are about 1000-fold higher than are obtained using conformationally incorrect L1 proteins which had been produced in E. coli. Therefore, this reference provides additional evidence that recombinant conformationally correct PV L1 proteins are effective immunogens which protect against PV infection. Additionally, the reference provides

evidence that this methodology is applicable to papillomavirus generically, since it teaches expression of an L1 protein from yet another HPV strain than was expressed by the present inventors.

Accordingly, based upon the above remarks, withdrawal of the outstanding §101 rejection of claims 15-18 and 20-24 is respectfully believed to be in order and is earnestly solicited.

Claims 1-26 and 41-45 were also rejected for failing to enable the invention. The Official Action contains five different bases for the rejection which are separately addressed below. First, the Examiner states that the disclosure does not enable the use of the subject recombinant L1 proteins to induce immunity against papillomavirus infection. This rejection is stated to be predicated upon the same reasons addressed supra in the §101 rejection. Accordingly, this rejection is respectfully traversed for the same reasons that the §101 rejection was traversed supra.

However, this rejection is additionally traversed as it pertains to claims 1-11 and 41-45. Kindly note that these claims are not directed to vaccine compositions or methods of vaccination. Rather, claims 1-11 are directed to a recombinantly produced L1 major capsid protein or antigenic fragment thereof which is capable of substantially reproducing the antigenicity of intact papillomas virions. Claims 41-45 are directed to the means for producing such L1 major capsid proteins by recombinant methods. Quite clearly the subject

application contains more than adequate evidence to establish that the disclosed L1 protein, which was expressed in cos cells, provides for substantially the same antigenicity as intact PV virions given the fact that it reacted with four different conformationally dependent monoclonal antibodies, and that these proteins may be used to detect the presence of neutralizing antibodies in sera.

The second basis of the §112 enablement rejection is the Examiner's belief that there is insufficient evidence in the application to establish that hosts such as cattle can elicit antibodies to L1 proteins which recognize conformational epitopes and thereby provide greater protection then anti-L1 antibodies recognizing linear epitopes.

The Action asserts that antisera obtained from rabbits is apparently the only host which provide evidence that conformationally correct L1 proteins afford greater protection then antibodies recognizing linear epitopes.

However, this rejection is respectfully traversed since it would appear to be improper. Example 1 contains explicit evidence that a large and significant reduction in cyst size was obtained for the sera of rabbits which were inoculated with intact BPV-1 or BPV-2 and steer antisera collected from animals which had been inoculated with homogenates of BPV-1 fibro-papillomas. Moreover, example 1 further establishes that rabbit sera, produced by immunization with intact BPV-1 and BPV-2, provided neutralizing titers of 10^6 and 10^4 respectively, while the hyperimmune steer sera comprised a

neutralizing titer of 10^6 to 10^3. Thus, contrary to the Office Action, there is evidence of record which indicates that antisera obtained from vaccinated cattle which recognize conformational epitopes of BPV-1 provide substantial protection against BPV-1.

The Official Action further asserts that there is no evidence that the subject L1 proteins are type specific for PV and will be useful for serological detection and typing of PV. However, this is vigorously disputed.

The HPV-1 protein expressed in cos cells has been shown to bind to four different antibodies specific to the HPV-1 virus (which recognize conformational epitopes). As discussed supra, such conformation dependent antibodies provide for neutralization of the papillomavirus by a host's immune system. Accordingly, it is abundantly clear that L1 proteins which bind conformation dependent antibodies will be well suited for the immunodetection of such neutralizing antibodies and as immunogens for inducing the production of such neutralizing antibodies.

The Office Action further states that the specification does not provide sufficient guidance as to which portions of the L1 protein are capable of reproducing the antigenicity of papillomavirus virions. In support of this rejection, two references by Stern and Berzovsky et al are cited which teach the inherent unpredictability in identifying antigenic epitopes even when the entire protein sequence is known.

It would appear that the Examiner believes that antigenic fragments are not enabled absent a specific characterization as to what specific sequences comprise the L1 conformational epitopes. However, it is respectfully submitted that such information is not required to enable the subject claims.

The subject claims are directed to any recombinant L1 protein or antigenic fragment which substantially reproduces the antigenicity of PV virions. Such recombinant L1 antigenic fragments are identified based upon their reactivity with conformation specific PV antibodies and their activity in the disclosed in vitro assays. Accordingly, given the information in this application, one skilled in the art could readily express the L1 protein of a desired papillomavirus, or a fragment thereof, express said protein or fragment in a suitable expression system such as cos cells, and screen the resulting expression products to identify those proteins capable of binding to conformation specific antibodies.

The Office Action further states that since immunity against PV is type specific, and since PV's induce greatly distinct disease conditions, that the disclosure contains inadequate evidence to support claims directed to L1 proteins and vaccines derived from any possible papillomavirus.

This rejection is also respectfully traversed. It is conceded that immunity against a specific papillomavirus strain will likely require administration of L1 proteins or neutralizing antibodies which are specific to the particular papillomavirus strain to which immunity is to be induced. It

is further acknowledged that only one (HPV-1) L1 protein was actually expressed in the subject application. However, this fact does not render the subject claims of undue scope.

As discussed supra, BPV-1 comprises a model system for studying papillomavirus antigenicity generically. Consequently, the evidence herein that conformationally correct L1 proteins provide for immunity to the BPV-1 viral strain is strongly suggestive that conformationally correct L1 proteins from other papillomaviruses will also confer protection.

With respect to the absence of specific L1 sequences from other papillomaviruses, it is respectfully noted that L1 proteins from the papillomavirus genus are highly conserved in structure. In support of this fact, applicants further attach to this Reply a listing of L1 sequences from a number of different papillomaviruses. Kindly note that these sequences comprise substantial sequence identity. Given this fact, one skilled in the art could readily use the subject L1 DNA sequences as hybridization probes to isolate the L1 gene from other papillomaviruses and express said L1 genes in e.g., suitable host cells mammalian cells.

Moreover, L1 proteins from all papillomaviruses are related in the fact that they similarly comprise the major capsid protein which is expressed on the surface of the corresponding papilloma virions. Accordingly, the corresponding papilloma virions could be used to prepare conformation specific antibodies which, in turn, may be used

to identify conformationally correct L1 proteins which reproduce the antigenicity of the corresponding papilloma virion.

Moreover, the general applicability of the claimed invention is further supported by Kirnbauer et al, Proc. Natl. Acad. Sci., 89:12180-12184, discussed supra, and attached to this Reply, which reference teaches that the L1 protein of another papillomavirus strain (HPV16), when expressed in correct conformation, comprises immunogenicity substantially equivalent to the intact infectious virions.

Finally, the Office Action states that the application contains inadequate evidence to establish that the claimed L1 proteins and antigenic fragments reproduce the antigenicity immunogenicity of the intact papillomavirus. It is believed that this rejection is moot at least in part given that the claims now recite that the L1 protein substantially reproduces the antigenicity of intact papillomavirus virions.

In response thereto, it is again respectfully noted that the subject L1 proteins mimic the antigenicity of the native L1 protein. This has been established by the fact that the disclosed L1 protein binds to four different conformation specific antibodies. Moreover, the L1 protein, since it is the major capsid protein of papillomavirus, is the papillomavirus protein which substantially (or entirely) induces antigenic response in a host.

Accordingly, the disclosure adequately establishes that the subject L1 proteins, which mimic the antigenicity of the

native L1 protein, substantially reproduces the antigenicity of intact papillomavirus virions.

Based upon the above remarks, withdrawal of the outstanding §112, first paragraph rejection of claims 1-26 and 41-45 is respectfully believed to be in order and is respectfully requested.

Claims 1, 10, 12-14, 18-21, 25 and 41 further stand rejected as allegedly being anticipated by Danos et al (U.S. Patent No. 4,551,270). This rejection is respectfully traversed.

Danos et al pertains to oligopeptides derived from the L1 and L2 proteins of HPV 1a type. Specifically, this patent teaches four oligopeptides which are six or eleven amino acids in length. These oligopeptides are purported to correspond to the antigenic epitopes of the virus. However, since the proteins were not actually synthesized, nor shown to bind or induce HPV 1a specific antibodies, whether the oligopeptides actually do correspond is unclear from the reference. The patent indicates that these oligopeptides may be synthesized by microbial expression or by chemical synthesis.

Therefore, Danos et al essentially teaches <u>linear oligopeptides</u> which are purported to be antigenic. In contrast, the subject application claims <u>conformationally correct L1 proteins which substantially reproduce the antigenicity of papilloma virions</u> (and which bind conformation specific antibodies). As discussed <u>supra</u>, this absolutely requires that the L1 proteins be expressed in a host system

which provides for the processing of L1 proteins into their native conformation such that the conformational epitopes are presented to a host immune system. However, Danos et al completely fails to teach expression of L1 proteins in a host system which would provide for proper conformation. Therefore, Danos et al fails to teach L1 proteins, as claimed, which substantially reproduce the antigenicity of intact papillomavirus virions.

Claims 1-26 and 41-45 have further been rejected under 35 U.S.C. §103 as being unpatentable over Christensen et al, Pilancinski et al, Sambrook et al and Danos et al.

Christensen et al pertains to monoclonal antibodies which recognize HPV 11, and which neutralize HPV-11 infection in the athymic mouse xenograft system.

Pilacinski et al teaches the cloning and expression of the BPV-1 L1 and L2 open reading frames in E. coli. as ß-galactosidase fusions. The reference notes that a number of antigenic sites were not presented to the host immune system after administration of these proteins and that the L1 protein may not be expressed in proper conformation.

Sambrook (The Molecular Cloning Manual) teaches that bacteria sometimes do not properly fold heterologous proteins and that one possible solution is to express such proteins in mammalian cells or insect cells (using baculovirus vectors).

Danos et al is discussed supra. Essentially, this reference pertains to linear oligopeptides from HPV 1a which are purported to be antigenic.

Essentially, the position of the Examiner is that it would have been obvious to have expressed the L1 protein in mammalian cells, given that Christensen et al teach that E. coli. fusion proteins do not present all the native L1 epitopes, and given that Sambrook teaches that mammalian proteins may produce proteins in native conformation, and further in view of the Christensen et al disclosure that conformational epitopes of PV provide for the induction of neutralizing antibodies. The use of the resultant L1 proteins as vaccines is asserted to be obvious in view of Danos et al.

This rejection is respectfully traversed. Contrary to the rejection, it was not expected that the L1 protein would be folded correctly when expressed in mammalian cells, or that this protein would reproduce the antigenicity of the intact PV virions. To the contrary, it is applicants' invention which provides the first evidence that the L1 protein, in and of itself, may provide protection against papillomavirus infection.

It was entirely possible that the proper conformation of the L1 protein would have absolutely required the L2 protein to be present. For example, the L2 protein might have been necessary to facilitate proper folding and processing of the L1 protein.

Furthermore, the L2 protein may itself have comprised conformational epitopes, or comprised parts of the conformational epitopes, which are necessary for antigenicity. While the evidence contained in the application indicates that

the L2 protein is not required for antigenicity of the L1 protein, this was not obvious prior to the present invention.

At best, it might have been obvious to have tried to express the L1 protein in mammalian cells in the hope of obtaining proteins which are folded properly and present the requisite conformational epitopes. However, as the Examiner is well aware, this is not the proper standard of patentability.

Moreover, the results obtained herein are repugnant to previous reports relating to papillomavirus antigenicity. As noted, the present inventors have provided convincing evidence that the L2 protein is not required for obtaining proteins which bind to conformationally correct neutralizing antibodies. However, this is in contrast to Zhou et al, J. Virol., (1991), 185:251-257, who reported that the L1 protein alone was incapable of inducing high-titer neutralizing antisera capable of preventing papillomavirus infection in vitro.

Moreover, the results herein would not have been obvious in light of a previous reference by Kajigaya et al, Proc. Natl. Acad. Sci., (1991), 88:4646-4650, relating to the human B19 parvovirus. This reference (which is discussed in Kirnbauer et al, Id.) reported that parvovirus VP2, by itself, was able to assemble, but that both VP1 and VP2 were required for induction of neutralizing antibodies. Accordingly, even assuming that the L2 protein was not required for proper

conformation, it may still have been essential for immunogenicity.

It is further noted that reported results pertaining to the polyoma/SV40 L1 expression system would not have suggested the efficacy of the present invention. The papillomaviruses are members of the A (PV) genus of the papoviridiae and the polyomaviruses comprise the B genus. However, Jenson et al, J. Natl. Cancer. Inst., (1980), 64, 495-500, teaches that these genera do not share any antigenic determinants on the major capsid protein and Law et al, (1979), 32, 199-207, teaches that these genera do not share polynucleotide sequence tumology.

Based on the known differences concerning the capsid proteins of these viruses, one skilled in the art would not have found the results pertaining to the polyoma/SV40 L1 system to be predictive of the efficacy of the claimed invention.

Further, even assuming for the sake of argument that expression of the L1 protein in mammalian cells would have been obvious, the rejection should be withdrawn in light of the unexpected results which are obtained by the subject invention.

The present inventors have established that conformationally correct L1 proteins provide for neutralizing antibody titers on the order of 10^6 and 10^4 (in rabbits) and 10^6 in cattle. This is in contrast to E. coli produced L1 proteins

which were only weakly antigenic and did not provide for high-titer neutralizing antisera.

The unexpected nature of these results are further supported by Kirnbauer et al, Proc. Natl. Acad. Sci., (1992) 189:12180-12184, who disclose that conformationally correct L1 proteins (expressed in insect cells) provided for antibody titers at least 1000-fold higher than achieved with previous E. coli derived L1 proteins, and that the results indicate that these proteins may induce long-term protection against papillomavirus infection. These unexpected results are in nowise suggested by the art.

In conclusion, it was not obvious from the art of record, and from what had been previously reported about papillomavirus immunity that the L1 protein, when expressed in the absence of the L2 and other viral proteins, would have provided for conformationally correct L1 proteins able to substantially reproduce the antigenicity of intact papillomavirus virions. Further, it was not obvious that such recombinant L1 proteins would provide for neutralizing antibody titers which are substantially greater (about three orders of magnitude better) than previous L1 proteins which were expressed in E. coli.

Accordingly, based upon the arguments set forth supra, withdrawal of the §103 rejection based on Christensen et al, Pilancinski et al, Sambrook et al, and Danos et al is respectfully requested.

From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such action is earnestly solicited.

If the Examiner has any questions regarding the subject application, he is respectfully requested to telephone the undersigned at 703-838-6612 so that prosecution of this application may be expedited.

Respectfully submitted,

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